High triacylglycerol turnover rate in human skeletal muscle

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In the present study we investigated the relationship between plasma fatty acids (FA) and intramuscular triacylglycerol (IMTAG) kinetics of healthy volunteers. With this aim [U-^{13}C]-palmitate was infused for 10 h and FA kinetics determined across the leg. In addition, the rate of FA incorporation into IMTAG in vastus lateralis muscle was determined during two consecutive 4-h periods (2–6 h and 6–10 h). Fifty to sixty per cent of the FA taken up from the circulation were esterified into IMTAG, whereas 32 and 42% were oxidized between 2–6 and 6–10 h, respectively. IMTAG fractional synthesis rate was $3.4 \pm 0.8\%\ h^{-1}$ and did not change between the two 4- h periods, despite an increase in arterial FA concentration (34%, $P < 0.01$). IMTAG concentration was also unchanged, implying that the IMTAG fractional synthesis rate was balanced by an equal rate of breakdown. FA oxidation increased over time, which could be due to the observed decline in plasma insulin concentration (−74%, $P < 0.01$). In conclusion, a substantial fraction of the fatty acids entering skeletal muscle in post-absorptive healthy individuals is esterified into IMTAG, due to its high turnover rate (29 h pool^{-1}). An increase in FA level, as a consequence of short-term fasting, does not seem to increase IMTAG synthesis rate and pool size.

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Fatty acids (FA) support a large portion of the energy requirement of resting skeletal muscle (Andres *et al.* 1956; Masoro, 1967; Dagenais *et al.* 1976; Abumrad *et al.* 1978). Indeed, under post-absorptive conditions there is a substantial fractional extraction of fatty acids by the skeletal muscle (Zierler, 1976), which makes this tissue an important determinant of whole body FA homeostasis. The two major fates of the fatty acids entering the myocyte are oxidation and esterification into complex lipids (Gorski, 1992). Intramuscular triacyclglycerol (IMTAG) represents the intracellular storage form of fatty acids and is potentially an important energy reservoir. Although the role of IMTAG as an energy source has been acknowledged in situations of augmented energy demand, such as prolonged exercise (Gorski, 1992; Watt *et al.* 2002), the role of IMTAG in resting conditions is less clear. In this regard, it is noteworthy that an increased IMTAG content is associated with insulin resistance and type 2 diabetes (Kelley *et al.* 2002; Hegarty *et al.* 2003), but why this occurs is still unknown. IMTAG is continuously synthesized and degraded and therefore the IMTAG content depends on the balance between these two processes. *In vitro* studies have shown a relationship between exogenous FA concentration and IMTAG synthesis rate (Budohoski *et al.* 1996; Dyck *et al.* 1997). However, the effects of an increased FA level on IMTAG turnover *in vivo* in humans at rest have not been directly investigated. This would also be of particular importance in the light of the evidence that an increased concentration of plasma FA in humans, induced by intravenous infusion of lipid and heparin, results within a few hours in an increase of IMTAG content (Boden *et al.* 2001; Brechtel *et al.* 2001) and in an impairment of insulin-stimulated glucose disposal (Bachmann *et al.* 2001; Boden *et al.* 2001). In order to clarify the mechanism leading to lipid accumulation in skeletal muscle it is important to study the relationship between plasma FA and IMTAG kinetics in this tissue. This was the primary aim of the present study, which investigated IMTAG turnover by means of the leg arterial–venous balance, stable isotopes and muscle biopsy techniques and defined the metabolic fate of blood-borne FA entering skeletal muscle of healthy individuals.

Methods

Subjects

Six healthy male volunteers (age 29 ± 2 years; weight 73 ± 3 kg; BMI $22.5 \pm 0.7\ kg\ m^{-2}$) participated in the study. Their body weight had been stable for at least the preceding 3 months and they were not on medication. The

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subjects were informed about the aim and the possible risks of the study and gave their written consent to participate. The study followed the Declaration of Helsinki and had been approved by the Ethical Committee of Copenhagen-Frederiksberg Communities.

Experimental procedure

Subjects refrained from any strenuous physical activity and from taking any substances known to affect lipid metabolism during the 48 h preceding the trial.

On the experimental day the subjects reported to the laboratory at 08.00 h after an overnight fast (12 h). After voiding and changing clothes, subjects rested in the supine position for 20 min. Teflon catheters (20G, Ohmeda, Wiltshire, UK) were then inserted using the Seldinger technique into the femoral vein (distal direction) and the femoral artery of one leg for blood sampling. The catheters were kept patent by flushing with 0.9% saline. An additional catheter was placed in a forearm vein for isotope infusion. After an additional 20 min of rest, the control samples were taken and femoral artery blood flow was measured by ultrasound Doppler (Radegran, 1997). Thereafter, a bolus of $NaH^{13}CO_3$ (1 μmol kg^{-1}) was given and a constant infusion of [U-^{13}C]-palmitate (0.0085 μmol kg^{-1} min^{-1}) started. The [U-^{13}C]-palmitate infusion was continued for 10 h, during which time the subjects remained in a supine position. Blood sampling and blood flow measurements were performed every 60 min throughout the experiment. Before blood sampling, a pneumatic cuff was placed under the knee and inflated to a suprasystolic pressure to avoid mixing of the femoral venous blood with blood from the lower leg (van Hall *et al.* 1999). After 2, 6 and 10 h from the start of the isotope infusion, a muscle biopsy was taken from the middle portion of the vastus lateralis muscle. The muscle specimen was immediately frozen in liquid nitrogen and stored at −80°C until further processing.

Analytical procedures

Blood for the measurement of substrate concentrations and isotopic enrichment was collected in pre-chilled tubes containing 0.3 M EDTA (10 μl (ml blood)$^{-1}$) and immediately centrifuged for 10 min at 1000 *g* and 4°C. The obtained plasma was immediately frozen and stored at −80°C until analyses. Additional blood was collected anerobically using heparinized syringes for the measurement of blood P_{O_2}, P_{CO_2}, pH, haemoglobin, oxygen saturation (ABL 700, Radiometer, Copenhagen, Denmark) and haematocrit. Plasma glucose concentration was determined flurometrically (Cobas Fara, Roche, Switzerland) and insulin was determined with an Elisa kit (Dako, Denmark).

Plasma FA were measured colourimetrically (NEFA-C kit, Wako Chemicals, Germany). Isolation of plasma FA for the determination of palmitate concentration and isotopic enrichment was performed according to Patterson *et al.* (1999). Palmitate concentration was measured by gas chromatography with FID (Autosystem XL, Perkin Elmer, Northwalk, USA), using heptadecanoic acid as internal standard. Palmitate ^{13}C-enrichment was measured by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS, Hewlett Packard 5890-Finnigan GC combustion III-Finnigan Delta^{plus}, Finnigan MAT, Germany) as previously described (van Hall *et al.* 2002). Palmitate enrichment was corrected by a factor of 17/16 to account for the extra methyl group of the methyl palmitate derivative.

For the measurement of the incorporation of plasma palmitate into plasma TAG, lipids were extracted with chloroform and methanol, and plasma TAG was isolated by thin layer chromatography. Plasma TAG was then hydrolysed and the TAG-FA were methylated as subsequently described for IMTAG. ^{13}C enrichment of palmitate in plasma TAG was then measured by GC-C-IRMS as described for plasma palmitate. Plasma TAG-palmitate concentration was determined by gas chromatography (Autosystem XL, Perkin Elmer, Northwalk, USA), using tripentadecanoin acid as internal standard. For the measurement of ^{13}C enrichment in arterial and venous blood CO_2, 1 ml blood was collected into 10 ml vacutainers and CO_2 was liberated into the headspace by adding 1 M H_2SO_4. $^{13}CO_2$ enrichments in blood were measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS, Delta^{plus}, Finnigan MAT, Bremen, Germany) as previously described (van Hall *et al.* 2002).

Muscle analysis

Muscle samples were freeze-dried and dissected free from blood and connective tissue and visible fat under a stereomicroscope. The muscle fibres were carefully freed from extramyocellular lipid contamination to assure the measurement of IMTAG and not total muscle TAG. The importance of such a procedure has been previously stressed (Guo, 2001). Intramuscular triacylglycerol concentration, intramuscular palmitate and IMTAG-palmitate enrichments and concentrations were measured as previously described (Sacchetti *et al.* 2002). Briefly, muscle lipids were extracted by chloroform–methanol and the organic phase divided for either IMTAG or intramuscular palmitate analysis. For the IMTAG analysis, the different lipid fractions were separated by thin layer chromatography and the triacylglycerol fraction was hydrolysed. The non-esterified FA fraction, isolated as described above for plasma FA, and the FA from IMTAG hydrolysis were methylated and their concentration and enrichment determined as described above for FA concentration and enrichment. IMTAG

concentration was calculated by adding the concentration of the six major fatty acids species in the IMTAG molecule (myristate, 14 : 0; palmitate, 16 : 0; palmitoleate, 16 : 1; stearate, 18 : 0; oleate, 18 : 1; linoleate, 18 : 2), since these represent more then 98% of the total fatty acids of human IMTAG (Andersson *et al.* 2000), and dividing this value by three.

Calculations

Leg FA balance, kinetics and oxidation. Net FA balance across the leg was calculated by multiplying the femoral arterial–venous concentration difference by plasma flow, computed as blood flow × (1 – haematocrit). Leg fractional extraction of palmitate was calculated as:

$$\text{Fractional extraction (\%)} = \frac{(C_a \times E_a) - (C_v \times E_v)}{C_a \times E_a} \times 100$$

where C_a and E_a, and C_v and E_v are the concentration and the tracer enrichment of palmitate (TTR) in the femoral artery and the femoral vein, respectively. Leg FA uptake was then calculated as:

$$\text{Uptake} = \text{Fractional extraction} \times C_a \times \text{plasma flow}$$

and palmitate release as:

$$\text{Release} = \text{uptake} - \text{net balance}$$

$$\text{Fraction palmitate uptake oxidized} = \frac{\frac{\{[(C_vCO_2 \times E_vCO_2) - (C_aCO_2 \times E_aCO_2)]/16\} \times \text{blood flow}}{[(C_a \times E_a) - (C_v \times E_v)] \times \text{plasma flow}}}{\text{ar}}$$

where C_aCO_2 and E_aCO_2, and C_vCO_2 and E_vCO_2, represent the concentration and the ^{13}C enrichment of blood CO_2 in the femoral artery and in the femoral vein, respectively, and ar is the fractional recovery of acetate across the leg. $^{13}CO_2$ production was divided by 16 in order to account for the fact that 1 mole of [U-^{13}C]-palmitate when oxidized gives 16 mol of $^{13}CO_2$. For the correction of plasma palmitate oxidation in the leg for isotopic exchange reactions, an acetate carbon recovery was used. This correction was made for each specific time point using the curve reported in a previous experiment for subjects resting in supine position for 9 h (Van Hall *et al.* 2002*a*) and considering the fact that in resting condition leg and whole body acetate recovery values are comparable (Mittendorfer *et al.* 1998). Finally, leg palmitate oxidation was calculated as the product of palmitate uptake and fraction of palmitate uptake oxidized. Plasma palmitate uptake, release and oxidation were converted to plasma FA uptake, release and oxidation by considering the fractional contribution of palmitate to total FA (26 ± 1%), as measured by gas chromatography.

IMTAG fractional synthesis rate (FSR) and rate of palmitate incorporation into IMTAG

The average fractional synthesis rate of IMTAG between the two 4-h periods, 2–6 h and 6–10 h, was calculated as the ratio between the difference in ^{13}C-palmitate enrichment in IMTAG at time 2 (t_2) and time 1 (t_1) and the average enrichment of the intramuscular palmitate over the same period of time, therefore considering the intramuscular FA as the precursor pool for IMTAG synthesis (Guo & Jensen, 1998; Sacchetti *et al.* 2002). Therefore:

$$\text{IMTAG FSR (\% h}^{-1}) = \frac{E_{\text{TAG-palmitate}}(t_2) - E_{\text{TAG-palmitate}}(t_1)}{(E_{\text{NEpalmitate}}(t_1) + E_{\text{NEpalmitate}}(t_2))/2} \times \frac{1}{4} \times 100$$

where $E_{\text{TAG-palmitate}}$ and $E_{\text{NEpalmitate}}$ is the ^{13}C enrichment of palmitate in IMTAG and of intramuscular non-esterified palmitate, respectively. The absolute rate of incorporation of plasma palmitate into IMTAG was calculated as the product of the IMTAG FSR and the IMTAG-palmitate pool size.

In order to calculate the proportion of the fatty acids taken up by the leg muscle destined for storage into IMTAG, the rate of palmitate esterification expressed per kilogram muscle mass was extrapolated to the total leg muscle of the leg (excluding the lower leg since during the time of the measurement the circulation was occluded by a pneumatic cuff placed under the knee). CO_2 content in arterial and venous blood was calculated according to Douglas *et al.* (1988). Total fat oxidation was calculated from legV_{O_2} and leg V_{CO_2} using stochiometric equations (Peronnet & Massicotte, 1991).

Statistical analysis

Data are expressed as means ± S.E.M. Differences between values at the different time points were analysed by using a repeated-measures analysis of variance (ANOVA). When ANOVA revealed a significant effect a Tukey *post hoc* test was used to isolate the differences. A paired *t* test was used to compare the average values during the 2–6 and 6–10 h periods. Significance was accepted at $P < 0.05$.

Results

After an overnight fast, the arterial FA concentration was 503 ± 61 μmol l^{-1} and increased significantly throughout the 10 h of the study to 1112 ± 102 μmol l^{-1} ($P < 0.001$) (Fig. 1*A*). The arterial FA concentration during the second 4-h period (6–10 h) was 38% higher compared with the first 4 h-period (2–6 h) ($P < 0.01$). Blood glucose was 4.90 mmol l^{-1} for the first 2 h after which it gradually declined to 4.55 mmol l^{-1} (Fig. 1*B*). At the start of the

experiment the insulin level was 15 ± 3.8 pmol l^{-1} which gradually decreased to 3.9 pmol l^{-1} over the 10 h (Fig. 1*B*).

Leg FA kinetics

Along with the increase in FA availability, FA fractional extraction across the resting leg decreased from 45% after 60 min to 22% at the end of the 10-h period ($P = 0.03$), being an average of 26% lower ($P < 0.01$) during the second 4 h-period (Fig. 2*A*). As a result, leg FA uptake did not show significant changes throughout the study period, averaging ~100–110 μmol l^{-1}. Leg FA release was also constant over time, with the tendency to be lower than the uptake (Fig. 2*B*).

Intramuscular FA and IMTAG

Despite the increasing arterial palmitate concentration, intramuscular palmitate content did not change over time (Table 1). The IMTAG content in the *vastus lateralis* muscle was also unchanged (Table 1),

Intramuscular enrichment of palmitate in IMTAG increased linearly over time ($P < 0.01$), whereas ^{13}C enrichment of intramuscular palmitate remained stable throughout the study period (Fig. 5). IMTAG fractional synthesis rate (FSR) was $3.4 \pm 0.4\%$ h^{-1} during the first 4- h period (4–6 h) and did not change significantly during the last 4 h (6–10 h) (Fig. 3).

The rate of palmitate incorporation in IMTAG was 0.9 ± 0.2 μmol (g dry muscle)$^{-1}$ h^{-1}, and was not significantly different between the two 4-h periods. The process of FA incorporation into IMTAG could account for approximately 50–60% of the leg FA uptake and was not significantly different between the two 4-h periods (Fig. 4). In contrast, leg FA oxidation was higher during the second 4-h period, which, with an unchanged uptake, implies that a higher fraction of the muscle FA uptake was directed towards oxidation (Fig. 4).

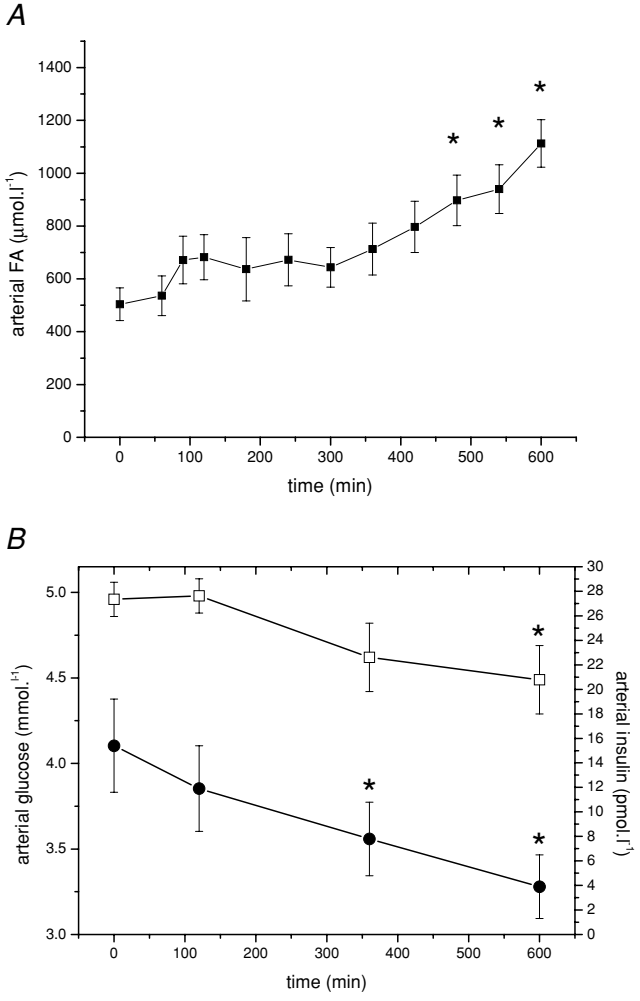

Figure 1. Arterial plasma FA (A), glucose and insulin (B) concentration during 10 h of rest in the post-absorptive state
*Significantly different from 0 min.

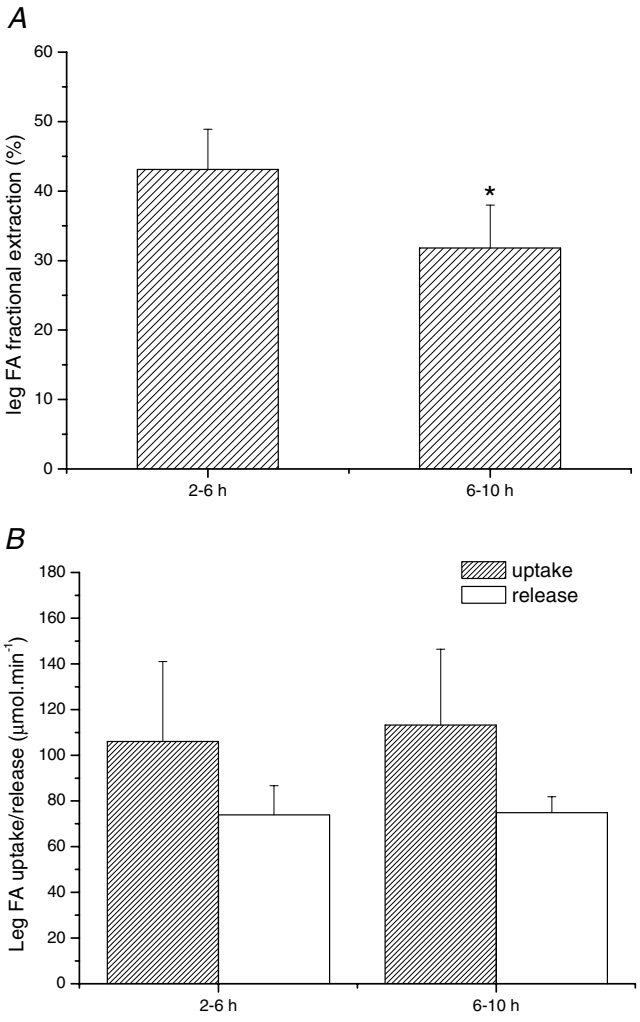

Figure 2. Leg plasma FA kinetics
FA fractional extraction (*A*) and FA uptake (*B*) and release in the leg.
*Significantly different from 2–6 h.

Table 1. Concentration of IMTAG and non-esterified palmitate (NE palmitate) in vastus lateralis muscle

	2 h	6 h	10 h
NE palmitate concentration (μmol (g dry wt)$^{-1}$)	0.334 ± 0.08	0.304 ± 0.10	0.410 ± 0.15
IMTAG concentration (μmol (g dry wt)$^{-1}$)	36.92 ± 11.2	41.28 ± 8.0	34.7 ± 6.0

Plasma TAG-palmitate enrichment and concentration

During the study, plasma ^{13}C-palmitate was progressively incorporated into plasma TAG (Fig. 5). The arterial enrichment of plasma TAG-palmitate was approximately 50% of the arterial non-esterified palmitate enrichment after 6 h and did not increase during the following 4 h. The venous plasma TAG-palmitate enrichment was not different between the artery and vein during the whole study period. Plasma TAG-palmitate concentration was stable during the study (Fig. 6). A difference between arterial and venous plasma TAG-palmitate concentration was not observed.

Discussion

Human skeletal muscle triacylglycerol (IMTAG) and FA kinetics were examined for 10 h with the following main findings: (1) approximately 50% of the plasma FA taken up by skeletal muscle is esterified into IMTAG and the remainder is oxidized; (2) the increase in FA concentration induced by short-term fasting did not increase IMTAG synthesis rate and did not lead to IMTAG and intramuscular FA accumulation in muscle; (3) the turnover rate of IMTAG in resting human skeletal muscle is high (turnover of the entire pool in 29 h).

IMTAG synthesis can account for a significant fraction of the FA entering resting skeletal muscle. As a matter of fact, when extrapolating the rate of FA incorporation into IMTAG in the muscle biopsy to the total leg muscle mass, it was estimated that 50–60% of the leg FA uptake from the circulation was directed towards esterification into the IMTAG pool. This is in agreement with previous studies performed on isolated rat muscle (Dyck *et al.* 1997, 2000) and emphasizes the importance of IMTAG turnover in skeletal muscle. The proportion of plasma FA taken up from the circulation and oxidized increased during the second 4-h period, whereas FA esterification rate into IMTAG was constant. In fact, IMTAG is usually thought not to contribute substantially to the total energy requirement of resting muscle when exogenous FAs are available (Dyck *et al.* 1997). Indeed, in the present study oxidation of blood-borne FA followed the total fat oxidation by the leg, also during the second 4 h-period, when reliance on fat for energy production increased (Table 2).

During a period of FA tracer infusion, label accumulation into plasma TAG has been previously reported (Diraison & Beylot, 1998). In order to account for the incorporation of FA into IMTAG originating not only from plasma FA but also from plasma TAG utilization, we measured ^{13}C-labelled palmitate incorporation into plasma TAG and its uptake by the leg. The enrichment of plasma TAG-palmitate increased over time, reaching an apparent steady state at a value about 50% lower than the arterial non-esterified palmitate enrichment. The percentage of TAG-palmitate of arterial palmitate enrichment after 4 h was remarkably similar to what was observed by Diraison & Beylot (1998) after 4 h of [1-C^{13}]-palmitate infusion in subjects fasted overnight. However, the present study clearly shows that the enrichment of palmitate in TAG does not reach arterial enrichment as could have been anticipated from that study. The reason for this could possibly be inherent in the different turnover rates of the VLDL-TAG fractions. A plasma TAG utilization by the leg was not detected, since both the arterial and femoral venous concentration and enrichment were similar. This implies that plasma TAG was not utilized by skeletal muscle, therefore providing no contribution to IMTAG synthesis and muscle FA oxidation. However, it should be recognized that a modest utilization of plasma TAG may fall below the analytical detection limit. Therefore, the possibility remains that, together with plasma FA, also FA originating from plasma TAG hydrolysis at the lumen of the capillaries could have contributed to some extent to the FA flux entering the muscle and directed towards oxidation or esterification into IMTAG.

IMTAG fractional synthetic rate in the vastus lateralis muscle was 3.4% h^{-1}. Interestingly, this value is comparable to that reported by other investigations with incubated rat muscles (Dyck *et al.* 1997, 2000, 2001). Moreover, here we report that the rate of synthesis of IMTAG in human muscle remains constant for a 10-h period in post-absorptive conditions, despite the rising arterial fatty concentration due to fasting. At first glance, this might seem to be in contradiction with the results of previous investigations conducted in rats showing a direct relationship between FA concentration in the medium and its rate of incorporation into IMTAG (Budohoski *et al.* 1996; Dyck *et al.* 1997). However, in the present study the increased availability of FA was not utilized by

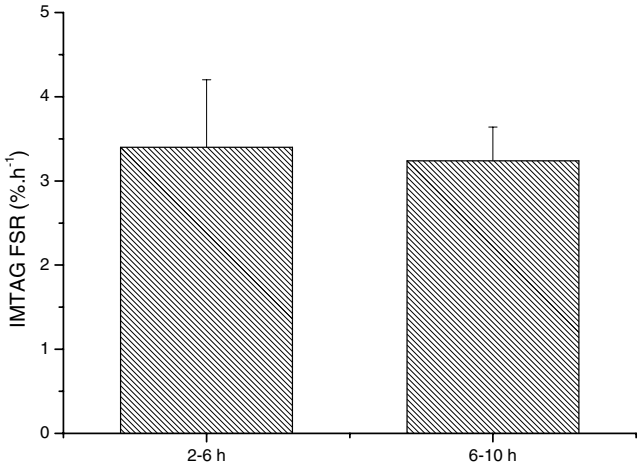

Figure 3. IMTAG synthesis rate
IMTAG fractional synthetic rate (FSR) during the first (2–6 h) and the second (6–10 h) 4-h period, separating two consecutive muscle biopsies.

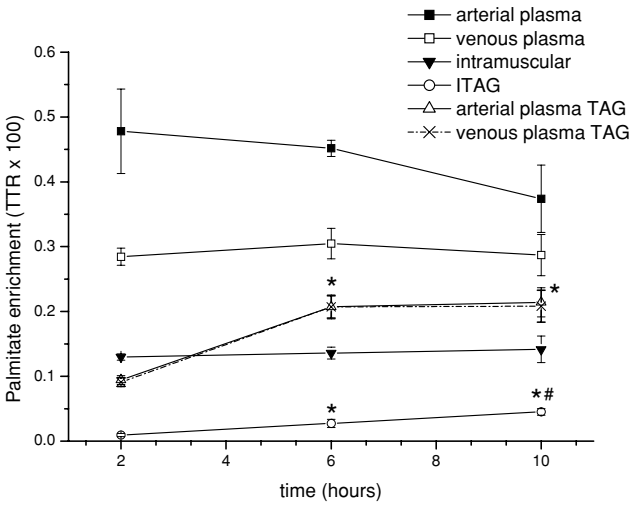

Figure 5. 13C-enrichment in plasma and muscle lipid pools
^{13}C-enrichment of plasma non-esterified palmitate, plasma TAG-palmitate, intramuscular palmitate and IMTAG-palmitate after 2, 6 and 10 h. *Significantly different from 2 h; . #Significantly different from 6 h.

skeletal muscle, as fractional extraction of FA by the leg was reduced and thus rate of influx of FA into the muscle was maintained. In addition, the increase in FA level induced by fasting in the present study seems to have a different effect on IMTAG synthesis compared with the one achieved by lipid/heparin infusion, which has been reported to induce an increase in IMTAG content (Boden *et al.* 2001). In that study, however, the lipid infusion protocol that induced an increase in FA level comparable with the one of the present study was associated with insulin infusion, which reduced FA oxidation and that might have resulted in a stimulation of IMTAG synthesis and in a reduction in IMTAG degradation, as demonstrated in isolated rat muscle (Dyck *et al.* 2000). This picture might mimic the situation present in type 2 diabetes, in which the combined increases in FA and insulin levels are coupled with a reduced capacity for FA oxidation (Kelley & Simoneau, 1994; Blaak *et al.* 2000), thereby potentially resulting in an elevated IMTAG synthesis rate. Differently, the increase in FA level resulting from a short period of fasting in the present study was instead reached in a situation where FA oxidation was increased and where the insulin level was declining, which may therefore explain the absence of a relationship

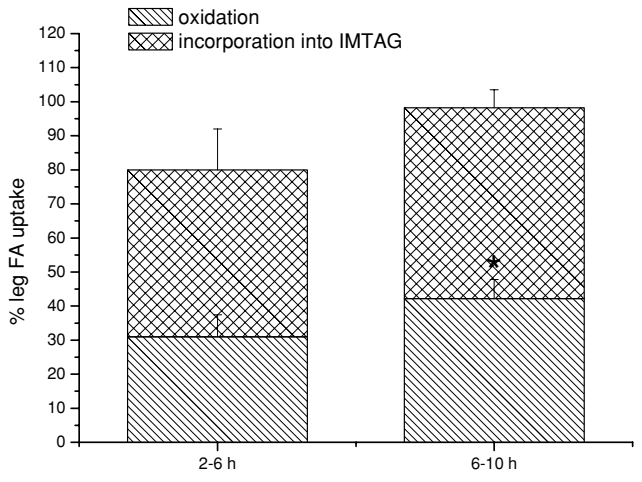

Figure 4. Muscle FA uptake towards esterification into IMTAG or oxidation
Intracellular partitioning of plasma FA taken up by the leg during the first and the second 4-h periods, separating two consecutive muscle biopsies. The total amount of palmitate incorporated into IMTAG was calculated by extrapolating the palmitate incorporation rate expressed per gram of resting muscle to the total leg muscle mass (upper leg only, since a cuff was used). *Significantly different from 2–6 h.

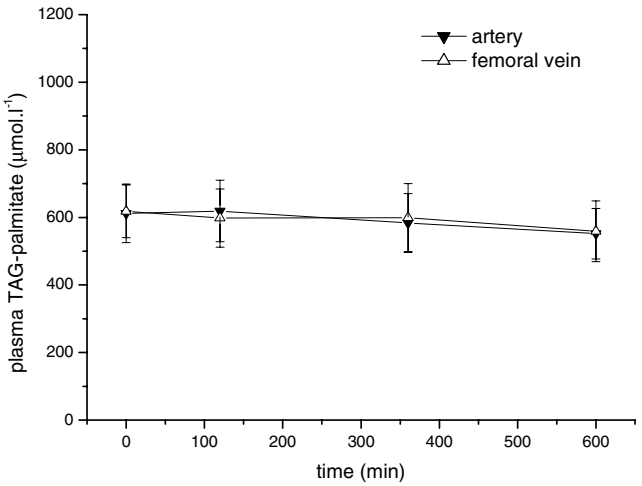

Figure 6. Plasma TAG-palmitate concentration
Plasma TAG-palmitate concentration in femoral artery and femoral vein after 2, 6 and 10 h.

Table 2. Average leg oxygen uptake and total fat oxidation during the two consecutive 4 h-periods

	2–6 h	6–10 h
Oxygen uptake (ml min^{-1})	30.5 ± 4.6	31.9 ± 3.1
Total fat oxidation (μmol FA min^{-1})	30.1 ± 4.3	48.6 ± 5.9*

*Significantly different from 2–6 h.

between increased FA level and IMTAG synthesis rate and pool size.

The situation might be different, however, when the fasting condition is maintained over several days, as it has been reported a net decrease in IMTAG content, associated to a substantially higher increase in FA level compared with that recorded in the present study, in volunteers who underwent 72 h of fasting (Stannard *et al.* 2002).

The IMTAG concentration in the vastus lateralis muscle did not change significantly during the study period, which implies that the synthesis rate was balanced by an equal rate of degradation. This means that IMTAG turnover in human resting skeletal muscle is a fast process, taking about 29 h. This picture is in contrast with the turnover rate of 3.3% over 24 h estimated in a recent study in humans (Hagstrom-Toft *et al.* 2002). However, in that study IMTAG turnover rate was not directly measured but estimated from glycerol release from the muscle, which, in turn, was obtained by extrapolating the venous concentration from the interstitial concentration measured by microdialysis. The validity of this estimation of IMTAG turnover is challenged by the evidence that glycerol metabolism takes place in skeletal muscle (Elia *et al.* 1993; Guo & Jensen, 1999; Jensen *et al.* 2001; van Hall *et al.* 2002).

In previous studies in isolated rat muscle using a pulse-chase, dual isotope technique, a loss of ^{14}C from prelabelled IMTAG was observed without a concomitant reduction in pool size (Dyck *et al.* 1997). Based on this, it has been suggested that small IMTAG subpools with high turnover rates (Dyck *et al.* 1997) may exist. The present data do not rule out the possibility of different FSRs arising from this phenomenon. Therefore the IMTAG turnover rate in the present study should be considered as an average estimate.

The two opposing and simultaneous processes of IMTAG synthesis and degradation are referred to as FA – TAG cycling, which, in light of the present findings, appears to be quite active in human skeletal muscle. With regard to the functional meaning of this substrate cycle, it has been originally proposed that this be inherent in the increase of sensitivity of FA mobilization in case of augmented energetic demand (Newsholme, 1977) or in the process of esterification in case of and increase in lipogenesis (Tagliaferro *et al.* 1990). Indeed, we have demonstrated that muscle contraction results in a reduction in IMTAG synthesis rate, which, together with an increased (or maintained) rate of IMTAG degradation contributes to an increased amount of intracellular FA to serve the augmented metabolic demand (Sacchetti *et al.* 2002). Along the same line, a high rate of IMTAG synthesis could be effective when the IMTAG pool needs to be rapidly reconstituted after being reduced, as for example after exercise (Larson-Meyer *et al.* 2002). This would also be in line with the observation that oxidative muscles, in which IMTAG utilization is higher during exercise (Baldwin *et al.* 1973; Hopp & Palmer, 1991), possess a higher rate of IMTAG synthesis at rest (Budohoski *et al.* 1996). Furthermore, an increased and sustained FA availability (such as the one obtained by lipid infusion), has been reported to result in an increase in IMTAG content within a few hours (Bachmann *et al.* 2001; Boden *et al.* 2001; Brechtel *et al.* 2001) which further underlines the highly dynamic nature of the IMTAG pool in resting muscle. It is also important to note that increased fat deposition is accompanied by an impairment of insulin sensitivity (Boden & Shulman, 2002; Hegarty *et al.* 2003), which emphasizes the importance of studying the relationship between fatty acid deposition and glucose disposal and utilization in muscle. Unfortunately, to date studies performing direct measurements of IMTAG turnover in skeletal muscle in humans with impaired glucose tolerance are lacking. What is clear, however, is that insulin resistance, type 2 diabetes and obesity are associated with an increased IMTAG content (Kelley *et al.* 2002). Nevertheless, IMTAG content has been found to be increased also in endurance trained subjects, who, on the contrary, have a high insulin sensitivity (Goodpaster *et al.* 2001). These two different metabolic conditions are characterized by a different capacity for lipid utilization in skeletal muscle, being high in endurance trained individuals (Saltin & Gollnick, 1983) and impaired in type 2 diabetic patients (Kelley & Simoneau, 1994). It could therefore be hypothesized that the metabolic consequences of intramuscular lipid accumulation are related to the capacity for fatty acid utilization in muscle. It has been suggested (Kelley & Goodpaster, 2001) that reduced turnover of the IMTAG stores might be associated with insulin resistance since athletes, who regularly lower and reconstitute their IMTAG stores during and after exercise, have enhanced insulin sensitivity. If this were the case, the high IMTAG turnover rate in healthy individuals found in the present investigation might play a role in the preservation of the optimal insulin sensitivity.

In conclusion, the present study indicates that IMTAG turnover in skeletal muscle of healthy individuals is high and that a major proportion of the FA entering the muscle from the circulation at rest is shunted toward esterification into IMTAG. The physiological elevation of blood-borne FA level as a consequence of a short period of fasting did

not influence the rate of IMTAG synthesis and did not increase IMTAG pool size.

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